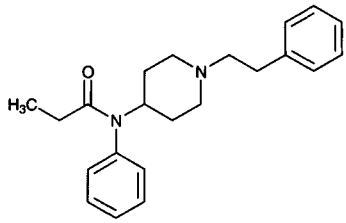
Fentanyl



Molecular formula: $C_{22}H_{28}N_2O$
Molecular weight: 336.48
CAS Registry No.: 437-38-7, 990-73-8 (citrate)
Merck Index: 4043
Lednicer No.: 1 299

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL 5 M NaOH + 100 μL MeCN + 600 μL hexane, mix, centrifuge at 2000 rpm for 5 min. Evaporate the organic phase under nitrogen at 30° for about 10 min. Reconstitute the residue in 100 μL MeCN:50 mM pH 3.0 phosphate buffer 50:50, inject a 50 μL aliquot. (Use silanized glassware.)

HPLC VARIABLES

Column: 100 × 8 4 μm cyano column (Waters)
Mobile phase: MeCN:50 mM pH 3.2 phosphate buffer 50:50
Flow rate: 2.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 6.2
Limit of detection: 150 pg
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: ampicillin, calcium chloride, clafoxan, diazepam, dobutamine, dopamine, furosemide, gentamicin, morphine, midazolam, pavulon, phenytoin, vitamin K

KEY WORDS

plasma

REFERENCE

Bansal,R.; Aranda,J.V. High-performance liquid chromatography microassay for the simultaneous determination of fentanyl and its major metabolites in biological samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 353–364.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL IS solution and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Prepare IS solution by adding 40 μL 1 mg/mL N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) in MeOH to 10 mL 100 mM NaH_2PO_4. Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM
Retention time: 11.4
Internal standard: N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) (14.5)

OTHER SUBSTANCES
Extracted: bupivacaine, mepivacaine, meperidine
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 2.8
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, doxepin, encainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: diphenhydramine, flecainide, nordoxepin, haloperidol (reduced), trazodone

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood, urine
Sample preparation: 50 μL Plasma or urine + 50 μL 4 M NaOH + 100 μL MeCN + 500 μL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: alfentanil, sufentanil

KEY WORDS
plasma

REFERENCE
Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 255.8

CHROMATOGRAM
Retention time: 14.202

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot

HPLC VARIABLES
Column: 100 × 3.9 4 μm Radial Pak phenyl (Waters)
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 phosphate buffer containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 11.6

OTHER SUBSTANCES
Simultaneous: degradation products, bupivacaine

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Tu,Y.H.; Stiles,M.L.; Allen,L.V.,Jr. Stability of fentanyl citrate and bupivacaine hydrochloride in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1990**, *47*, 2037–2040.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 7.88
Internal standard: haloperidol (9.74)

OTHER SUBSTANCES
Extracted: sufentanil

KEY WORDS
injections

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

SAMPLE
Matrix: formulations

Sample preparation: Dilute 5-fold with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 KH_2PO_4 containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 5.1

OTHER SUBSTANCES
Noninterfering: midazolam

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Bhatt-Mehta,V.; Johnson,C.E.; Leininger,N.; Agarwal,M. Stability of fentanyl citrate and midazolam hydrochloride during simulated intravenous coadministration, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 511–513.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL DMSO, vortex, centrifuge, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was 1 M ammonium acetate. B was MeCN:MeOH:THF:1 M ammonium acetate 30:20:40:10. A:B from 100:0 to 35:65 over 40 min.
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; pharmacokinetics

REFERENCE
Tateishi,T.; Wood,A.J.J.; Guengerich,F.P.; Wood,M. Biotransformation of tritiated fentanyl in human liver microsomes. Monitoring metabolism using phenylacetic acid and 2-phenylethanol, *Biochem.Pharmacol.*, **1995**, *50*, 1921–1924.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 μBondapak C18
Mobile phase: MeCN:water adjusted to pH 3 with phosphoric acid 70:30
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Internal standard: codeine

REFERENCE
Vanbever,R.; Le Boulengé,E.; Préat,V. Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors, *Pharm.Res.*, **1996**, *13*, 559–565.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.46

OTHER SUBSTANCES
Simultaneous: tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, buprenorphine, dextromoramide, phenoperidine, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine,

chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 9.39
Limit of detection: 5.5 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, nalbuphine, ethylmorphine, morphine, codeine, meperidine, tramadol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine
Interfering: butorphanol

REFERENCE

Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, *570*, 339–350.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.40 (A), 6.03 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

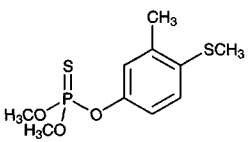
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Fenthion



Molecular formula: $C_{10}H_{15}O_3PS_2$
Molecular weight: 278.33
CAS Registry No.: 55-38-9
Merck Index: 4044

SAMPLE

Matrix: blood

Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 μL MeOH:water 70:30, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 μA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 μm, collector slit 400 μm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, positive-ion mode

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CHROMATOGRAM
Retention time: 10.6
Limit of detection: 20 ng

OTHER SUBSTANCES
Extracted: diazinon, dichlorvos, dimethoate, dimethylvinphos, ediphenphos, IBP, isoxathon, malathion, phenthoate, propaphos, pyridafenthion

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KEY WORDS
serum; SPE; m/z 279

REFERENCE
Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in 1 mL 0.5 M NaOH, heat at 75° for 45 min, cool, add 2 drops methyl isobutyl ketone, add 2 drops 0.1% dansyl chloride in acetone, shake well, heat at 65° for 30 min, cool, acidify with 10% HCl, add 300 μL benzene (Caution! Benzene is a carcinogen!), extract, inject a 1-10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:MeOH 95:5
Flow rate: 0.78
Injection volume: 1-10
Detector: F ex Turner filter no. 811 em Turner filter no. 817

CHROMATOGRAM
Retention time: 3

KEY WORDS
derivatization; normal phase

REFERENCE
Frei,R.W.; Lawrence,J.F. Fluorigenic labelling in high-speed liquid chromatography, *J.Chromatogr.*, **1973**, *83*, 321–330.

SAMPLE
Matrix: solutions
Sample preparation: Condition a 10 × 2 SPE column packed with 40 μm octadecylsilica (Spark Holland) with 10 mL MeCN, 10 mL MeOH, and 10 mL water at 2 mL/min. Add nitric acid to a final concentration of 0.5% to water sample, filter (0.45 μm), add a 150 mL aliquot to the SPE column at 3 mL/min, wash with 3 mL distilled water, elute the contents of the SPE column on to the analytical column with the mobile phase.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 60 RP-8 endcapped C8 (Merck)
Mobile phase: Gradient. A was MeCN:MeOH 80:20. B was water. A:B from 10:90 to 40:60 over 10 min, maintain at 40:60 for 5 min, to 90:10 over 33 min, return to initial conditions over 5 min.
Flow rate: 1
Detector: UV 220

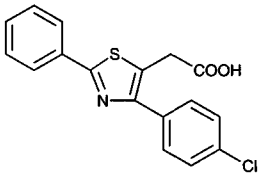
CHROMATOGRAM
Retention time: 39.8
Limit of detection: 65 ng/L

OTHER SUBSTANCES
Simultaneous: azinphos-ethyl, azinphos-methyl, chlorfenvinphos, dichlorvos, fenitrothion, malathion, mevinphos, parathion-ethyl, parathion-methyl
Interfering: diazinon

KEY WORDS
groundwater; wastewater; SPE

REFERENCE
Lacorte,S.; Barceló,D. Improvements in the determination of organophosphorus pesticides in ground- and wastewater samples from interlaboratory studies by automated on-line liquid-solid extraction followed by liquid chromatography-diode array detection, *J.Chromatogr.A*, **1996**, *725*, 85–92.

Fentiazac



Molecular formula: $C_{17}H_{12}ClNO_2S$
Molecular weight: 329.81
CAS Registry No.: 18046-21-4
Merck Index: 4045
Lednicer No.: 4 96

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 13.49
Limit of detection: <120 ng/mL

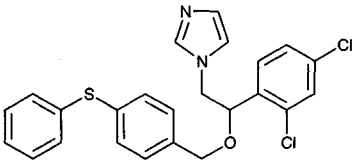
KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Fenticonazole



Molecular formula: $C_{24}H_{20}Cl_2N_2OS$
Molecular weight: 455.41
CAS Registry No.: 72479-26-6, 73151-29-8 (nitrate)
Merck Index: 4047
Lednicer No.: 4 93

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 2 mL dichloromethane, elute with 3 mL MeOH:buffer 85:15. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH,

inject 20 μL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 32
Internal standard: ketoconazole (7)

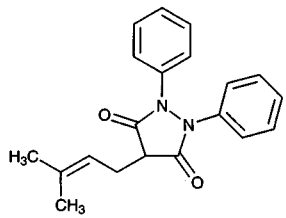
OTHER SUBSTANCES
Simultaneous: clotrimazole, ketoconazole, bifonazole, tioconazole, isoconazole, econazole, miconazole

KEY WORDS
tablets; creams

REFERENCE
Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

Feprazone

Molecular formula: $C_{20}H_{20}N_2O_2$
Molecular weight: 320.39
CAS Registry No.: 30748-29-9
Merck Index: 4053



SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 250 μL 1 M HCl + 5 mL diethyl ether, mix, centrifuge at 1100 g for 10 min. Remove the organic phase, repeat the extraction, combine the organic extracts, evaporate under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 20 μL aliquot. Urine. 1 mL Urine + 250 μL 1 M HCl + 5 mL diethyl ether, rotate for 15 min. Remove the organic layer, add 1 mL 1% sodium hydrogen carbonate, vortex for 1 min. Remove the organic layer and evaporate it under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil
Mobile phase: MeCN:water:acetic acid 40:59.4:0.6
Flow rate: 0.8
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 8.3
Internal standard: feprazone

OTHER SUBSTANCES
Extracted: tiaprofenic acid (UV 310)

Noninterfering: alclofenac, diclofenac, fenoprofen, flunixin, flurbiprofen, ibuprofen, indomethacin, naproxen, oxyphenbutazone, phenylbytazone, piroxicam

KEY WORDS
plasma; feprazone is IS

REFERENCE
Delbeke,F.T.; Baert,K.; De Backer,P. Disposition of human drug preparations in the horse. VI. Tiaprofenic acid, *J.Chromatogr.B*, **1997**, *704*, 207–214.

Fibrinolysin

Molecular weight: about 90000
CAS Registry No.: 9004-09-5, 9001-90-5
Merck Index: 7678

SAMPLE
Matrix: solutions

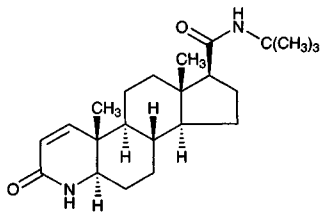
HPLC VARIABLES
Column: 100 × 6 Asahipak GS-520-AHA-ABA (Prepare by suspending 10 g Asahipak-GS gel (Asahi Chemical Industry) in water, sonicate for 5 min, wash with 200 mL water, wash with 200 mL dioxane, suspend in 100 mL dioxane, add 3.24 g 1,1'-carbonyldiimidazole, stir gently for 15 min at room temperature, wash with 200 mL dioxane, suspend in 200 mL 1 M sodium bicarbonate containing 1 M 6-aminohexanoic acid, shake at 4° for 25 h, wash with 200 mL water, wash with 100 mL 1 M NaCl, wash with 200 mL water. Suspend 2 g of the gel in 15 mL 200 mM pH 4.752-(morpholino)ethanesulfonic acid/NaOH buffer, add 288 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride, stir gently for 30 min, add 28.3 mg p-aminobenzamide monohydrochloride, adjust pH three times to 4.75 with 1 M HCl or 1 M NaOH at 30 min intervals, shake gently at room temperature for 24 h, wash with 150 mL water, wash with 100 mL 50 mM NaOH containing 1 M NaCl, wash with 100 mL 50 mM HCl containing 1 M NaCl, wash with water until washings are neutral. Caution! Dioxane is a carcinogen. It may be possible to use acetone instead.)
Mobile phase: Gradient. Buffer A, after 10 min buffer B, after 25 min buffer B containing 40 mM 6-hexanoic acid, after 40 min buffer B containing 40 mM 6-hexanoic acid and 1 M urea (step gradient). Buffer A was 50 mM pH 6.5 sodium phosphate. Buffer B was 50 mM pH 7.4 sodium phosphate containing 100 mM NaCl.
Flow rate: 1.8
Detector: F ex 285 em 340 (?)

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Simultaneous: plasminogens

REFERENCE
Ito,N.; Noguchi,K.; Kazama,M.; Shimura,K.; Kasai,K.-I. Separation of human Glu-plasminogen, Lys-plasminogen and plasmin by high-performance affinity chromatography on Asahipak GS gel coupled with *p*-aminobenzamide, *J.Chromatogr.*, **1985**, *348*, 199–204.

Finasteride



Molecular formula: $C_{23}H_{36}N_2O_2$
Molecular weight: 372.55
CAS Registry No.: 98319-26-7
Merck Index: 4125
Lednicer No.: 5 49

SAMPLE

Matrix: blood
Sample preparation: Condition a 6 mL 300 mg Carbograph SPE cartridge (Alltech) with 2 mL water and 4 mL MeOH. Add 100 µL 10 µg/mL IS to 1.0 mL plasma, briefly vortex, add the mixture to the SPE cartridge. Wash with 2 mL water and 4 mL MeOH:water 20:80. Elute with 2 mL MeOH:chloroform 20:80 (Caution! Chloroform is a carcinogen!). Centrifuge eluate at 1000 g for 10 min, filter through a WTP 500 nm filter, evaporate to dryness with a stream of nitrogen under vacuum. Reconstitute the residue in 1.0 mL mobile phase, vortex, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 40 µm Pelliguard (Supelco)
Column: 250 × 4.6 5 µm reversed-phase Symmetry (Waters)
Mobile phase: MeCN:40 mM pH 4.0 orthophosphoric acid 45:55
Flow rate: 1.2
Injection volume: 50
Detector: UV 215

CHROMATOGRAM

Retention time: 14.6
Internal standard: 4-androstene-3,17-dione (9.8)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Carlucci,G.; Mazzeo,P. Finasteride in biological fluids: extraction and separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 245–248.

SAMPLE

Matrix: blood
Sample preparation: Non-buffered extraction. 1 mL Plasma + 100 µL 100 ng/mL IS in MeOH. Add 7 mL MTBE, mix and rotate for 15 min, centrifuge, remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 300 µL mobile phase, inject an aliquot. Buffered extraction. 1 mL Plasma + 100 µL 100 ng/mL IS in MeOH. Add 1 mL 200 mM pH 9.8 carbonate buffer and 7 mL MTBE. Extract as described above. Inject an aliquot.

HPLC VARIABLES

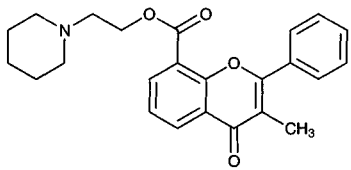
Column: 30 × 4.6 3 µm BDS Hypersil C18 (A), 50 × 2 3 µm BDS Hypersil C18 (B)
Mobile phase: MeCN:water containing 0.1% formic acid 90:10
Column temperature: 60
Flow rate: 1(A), 0.2 (B)
Detector: MS, PE Sciex API IIIplus tandem MS, heated nebulizer 500°, corona discharge needle +4 µA, APCI positive ion, nebulizing gas air 550 kPa, auxiliary gas at 2 mL/min, curtain gas nitrogen at 0.9 mL/min, orifice +50 V, interface heater 60°, collision gas argon, m/z 373 (A), Turbo ion spray, nebulizing gas air 410 kPa, auxiliary gas at 6 mL/min, curtain gas at 1mL/min, orifice +30 V, interface heater 60°, m/z 373 (B)

CHROMATOGRAM
Retention time: 0.5 (A), 1 (B)
Internal standard: finasteride analog (1.1)
Limit of detection: 25 pg/mL (B)

KEY WORDS
plasma

REFERENCE
Matuszewski,B.K.; Constanzer,M.L.; Chavez-Eng,C.M. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: A method for determination of finasteride in human plasma at picogram per milliliter concentrations, *Anal.Chem.*, **1998**, *70*, 882–889.

Flavoxate



Molecular formula: $C_{24}H_{25}NO_4$
Molecular weight: 391.47
CAS Registry No.: 15301-69-6, 3717-88-2 (HCl)
Merck Index: 4135
Lednicer No.: 2 392

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortrip-

tyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.30 (A), 5.94 (B)

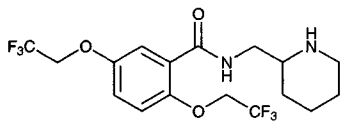
KEY WORDS

also details of plasma extraction; also acebutolol; acepromazine; acetaminophen; acetazolamide; acetophenazine; albuterol; alprazolam; amitriptyline; amobarbital; amoxapine; antipyrine; atenolol; atropine; azatadine; baclofen; benzocaine; bromocriptine; brompheniramine; brotizolam; bupivacaine; buspirone; butabarbital; butalbital; caffeine; carbamazepine; cetirizine; chlorcyclizine; chlordiazepoxide; chlormezanone; chloroquine; chlorpheniramine; chlorpromazine; chlorpropamide; chlorprothixene; chlorthalidone; chlorzoxazone; cimetidine; cisapride; clomipramine; clonazepam; clonidine; clozapine; cocaine; codeine; colchicine; cyclizine; cyclobenzaprine; dantrolene; desipramine; diazepam; diclofenac; diflunisal; diltiazem; diphenhydramine; diphenidol; diphenoxylate; dipyridamole; disopyramide; dobutamine; doxapram; doxepin; droperidol; encainide; ethidium bromide; ethopropazine; fenoprofen; fentanyl; fluoxetine; fluphenazine; flurazepam; flurbiprofen; fluvoxamine; furosemide; glutethimide; glyburide; guaifenesin; haloperidol; homatropine; hydralazine; hydrochlorothiazide; hydrocodone; hydromorphone; hydroxychloroquine; hydroxyzine; ibuprofen; imipramine; indomethacin; ketoconazole; ketoprofen; ketorolac; labetalol; levorphanol; lidocaine; loratadine; lorazepam; lovastatin; loxapine; mazindol; mefenamic acid; meperidine; mephenytoin; mepivacaine; mesoridazine; metaproterenol; methadone; methdilazine; methocarbamol; methotrexate; methotrimeprazine; methoxamine; methyldopa; methylphenidate; metoclopramide; metolazone; metoprolol; metronidazole; midazolam; moclobemide; morphine; nadolol; nalbuphine; naloxone; naphazoline; naproxen; nifedipine; nizatidine; norepinephrine; nortriptyline; oxazepam; oxycodone; oxymetazoline; paroxetine; pemoline; pentazocine; pentobarbital; pentoxifylline; perphenazine; pheniramine; phenobarbital; phenol; phenolphthalein; phentolamine; phenylbutazone; phenyltoloxamine; phenytoin; pimozide; pindolol; piroxicam; pramoxine; prazepam; prazosin; probenecid; procainamide; procaine; prochlorperazine; procyclidine; promazine; promethazine; propafenone; propantheline; propiomazine; propofol; propranolol; protriptyline; quazepam; quinidine; quinine; racemethorphan; ranitidine; remoxipride; risperidone; salicylic acid; scopolamine; secobarbital; sertraline; sotalol; spironolactone; sulfinpyrazone; sulindac; temazepam; terbutaline; terfena-

dine; tetracaine; theophylline; thiethylperazine; thiopental; thioridazine; thiothixene; timolol; tocainide; tolbutamide; tolmetin; trazodone; triamterene; triazolam; trifluoperazine; trifluopromazine; trimeprazine; trimethoprim; trimipramine; verapamil; warfarin; xylometazoline; yohimbine; zopiclone

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Flecainide



Molecular formula: $C_{17}H_{20}F_6N_2O_3$
Molecular weight: 414.35
CAS Registry No.: 54143-55-4, 54143-56-5 (acetate)
Merck Index: 4136
Lednicer No.: 3 59

SAMPLE

Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 µL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 µL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 µL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 3.1
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, encainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: diphenhydramine, doxepin, fentanyl, haloperidol, nordoxepin, trazodone

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 299

CHROMATOGRAM
Retention time: 5.22
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvox-

amine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Homogenize 15 g liver with water to a final volume of 90 mL, dilute 1 to 10 with water. Homogenize gastric contents, dilute 1 to 100 with water. Dilute urine 1 to 25. 1 mL Whole blood, diluted liver homogenate, diluted gastric contents, or diluted urine + 50 μL 100 μg/mL clomipramine, mix, inject an aliquot.

HPLC VARIABLES

Guard column: 20 mm long Hypersil ODS

Column: 150 mm long Hypersil ODS

Mobile phase: MeCN:10 mM Na_2HPO_4:n-nonylamine 40:60:0.12, pH 3

Flow rate: 1

Detector: UV 295

CHROMATOGRAM

Retention time: 3

Internal standard: clomipramine (5.5)

Limit of quantitation: 1 μg/mL

KEY WORDS

liver; whole blood

REFERENCE

Sadler,D.W.; Quigley,C. Unsuspected self-poisoning with flecainide and alcohol, *J.Forensic Sci.*, **1995**, *40*, 903–905.

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute urine 1:100. 1 mL Plasma or diluted urine + 100 μL 4 μg/mL IS in water + 1 mL water + 1 mL 1 M NaOH + 8 mL distilled diethyl ether, shake for 15 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL freshly prepared 49 mM 1-[(4-nitrophenyl)sulfonyl]-L-prolyl chloride in ethyl acetate, add 100 μL 0.016% triethylamine in ethyl acetate, vortex for 5 s, heat at 80° for 2 h, cool, wash with 3 mL 600 mM HCl, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40-180 μL aliquot. (Synthesis of 1-[(4-nitrophenyl)sulfonyl]-L-prolyl chloride is as follows. Stir 40-45 mmoles L-(-)-proline in 40 mL THF and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrophenylsulfonyl chloride in 40 mL THF dropwise, heat at 50° for 3 h and maintain at pH 8 or above, cool, acidify to pH 2 and extract into chloroform. Extract with 10% potassium carbonate, acidify the aqueous layer, extract into chloroform. Dry the chloroform extract, evaporate, recrystallize from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles of the 4-nitrophenylsulfonylproline in 100 mL benzene under reflux condenser fitted with a calcium sulfate drying tube, add dropwise a five-fold molar excess of thionyl chloride in 50 mL benzene, heat at 35-40° until formation of the acid chloride is complete (about 48 h, monitor by IR spectroscopy), evaporate, recrystallize product from HPLC-grade heptane (mp 110-110.5°).)

HPLC VARIABLES

Guard column: 50 mm long octadecyl Pellicular ODS (Whatman)

Column: 300 × 3.9 Bondapak C18

Mobile phase: MeCN:water:triethylamine 45:55:0.2

Flow rate: 1

Injection volume: 40-180
Detector: UV 280

CHROMATOGRAM
Retention time: 29.8 (R), 31.9 (S)
Internal standard: (R,S)-N-(2-piperidylmethyl)-2,3-bis-(2,2,2-trifluoroethoxy)benzamide (R, 25.5, S, 27.9)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; derivatization; pharmacokinetics; chiral

REFERENCE
Alessi-Severini,S.; Jamali,F.; Pasutto,F.M.; Coutts,R.T.; Gulamhusein,S. High-performance liquid chromatographic determination of the enantiomers of flecainide in human plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 257–260.

SAMPLE
Matrix: formulations
Sample preparation: Extract ground tablets containing 10-50 mg of the compound with 100 mL MeOH, filter. Add 500 μL 100 μg/mL IS in MeOH to 1 mL filtrate, make up to 10 mL with MeOH. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb
Mobile phase: MeCN:MeOH:buffer 30:60:10 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.30 (flecainide acetate)
Internal standard: amiodarone (7.46)
Limit of quantitation: 4 μg/mL

KEY WORDS
tablets

REFERENCE
Paw,B.; Przyborowski,L.; Slawik,T. Determination of flecainide acetate in tablets by HPLC and UV-spectrophotometry, *Pharmazie*, **1998**, *53*, 97–98.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES
Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM
Retention time: k' 2.93
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, ephedrine, methamphetamine, propranolol

KEY WORDS
derivatization; complexation

REFERENCE
Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18 (A) or 200 × 4.6 5 μm Nucleosil cyano (B)
Mobile phase: MeOH:water:THF 62:35:3 (A) or n-hexane:isopropanol:diethylamine 95:5:0.05 (B)
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 29.0 (R (A)), 31.0 (S (A)), 22.2 (R (A)), 26.8 (S (B))

OTHER SUBSTANCES
Simultaneous: mexiletine (system A only)

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Spherisorb S5SCX
Mobile phase: MeOH:MeCN:water 40:40:20 containing 25 mM perchloric acid
Flow rate: 2
Detector: F ex 215 no emission filter

CHROMATOGRAM
Retention time: 4.5
Internal standard: benzimidazole (7)

OTHER SUBSTANCES
Simultaneous: propranolol

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 µL of a 1 mM solution in MeOH or water with 50 µL pH 8 borate buffer and 50 µL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 µL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 µL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl, extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

HPLC VARIABLES
Column: 250 × 4 5 µm Zorbax-SIL
Mobile phase: n-Hexane:isopropanol 100:1.5
Flow rate: 1.5
Injection volume: 100
Detector: UV 230, F ex 270 em 365

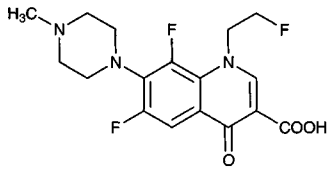
CHROMATOGRAM
Retention time: k' 14.4 (R-(-)), k' 15.2 (S-(+))

OTHER SUBSTANCES
Simultaneous: metoprolol, tocainide
Interfering: propafenone

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

Fleroxacin



Molecular formula: $C_{17}H_{18}F_3N_3O_3$
Molecular weight: 369.34
CAS Registry No.: 79660-72-3
Merck Index: 4137
Lednicer No.: 5 125

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Dilute urine 1:20. Dilute bile 1:10. 500 μL Serum, diluted urine, or diluted bile + 3.2 mL dichloromethane, vortex, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove 3 mL of the lower organic phase and add it to 200 μL 100 mM NaOH, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:buffer 10:90, pH adjusted to 2 with 14.6 M phosphoric acid (Buffer was 10 mM NaH_2PO_4 containing 5 mM tetrabutylammonium bromide.)
Flow rate: 2
Injection volume: 20
Detector: F ex 277 em 445

CHROMATOGRAM

Retention time: 3
Limit of detection: 25 ng/mL (bile), 50 ng/mL (urine), 2.5 ng/mL (serum)

OTHER SUBSTANCES

Noninterfering: amikacin, aztreonam, carbamazepine, cephalosporins, ciprofloxacin, clavulanic acid, difloxacin, digitoxin, digoxin, fosfomycin, furosemide, gentamycin, imipenem, lidocaine, netilmicin, norfloxacin, pefloxacin, penicillins, phenobarbital, phenytoin, primidone, procainamide, quinidine, rifampin, salicylic acid, teicoplanin, temafloxacin, theophylline, tobramycin, valproic acid, vancomycin
Interfering: ofloxacin

KEY WORDS

serum; human; rabbit

REFERENCE

Koechlin,C.; Jehl,F.; Linger,L.; Monteil,H. High-performance liquid chromatography for the determination of three new fluoroquinolones, fleroxacin, temafloxacin and A-64730, in biological fluids, *J.Chromatogr.*, **1989**, *491*, 379–387.

SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 22:78 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 287

CHROMATOGRAM
Retention time: 4.55
Internal standard: difloxacin (9.5)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL 7% perchloric acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Flow rate: 0.8
Injection volume: 20
Detector: F ex 288 em 475 bandpass filter

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Griggs,D.J.; Wise,R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum, *J.Antimicrob.Chemother.*, **1989**, *24*, 437–445.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.2 μm cellulose acetate, Schleicher & Schuell) while centrifuging at 4° at 2500 g for 5 min, maintain filtrate at 4°, inject a 5 μL aliquot of the filtrate onto column A and elute to waste with mobile phase A, collect the effluent containing fleroxacin in a sample loop and backflush the contents of this loop onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 40 × 6 12 μm TSKgel PWxl methacrylate polymer gel (Toso-Haas); B 250 × 4.6 5 μm Zorbax Rx-C8
Mobile phase: A Isopropanol:30 mM pH 6.2 potassium phosphate buffer 10:90; B MeCN:50 mM pH 2.7 potassium phosphate buffer 18:82
Flow rate: A 0.6; B 1
Injection volume: 5
Detector: UV 287

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 270 ng/mL

OTHER SUBSTANCES
Extracted: Ro 23-9424 (a pro-drug)

KEY WORDS
plasma; column-switching; heart-cut; pharmacokinetics

REFERENCE
Szuna,A.J.; Blain,R.W. Determination of a new antibacterial agent (Ro 23-9424) by multidimensional high-performance liquid chromatography with ultraviolet detection and direct plasma injection, *J.Chromatogr.*, **1993**, *620*, 211–216.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 20 μL 30 μg/mL pipemidic acid + 250 μL 25% sodium sulfate, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the lower organic layer and add it to 200 μL 1 M NaOH, shake fast for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of detection: 50 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N.; Wong,M.; Stragand,L.; Puleo,C. Effect of a fat- and calcium-rich breakfast on pharmacokinetics of fleroxacin administered in single and multiple doses, *Antimicrob.Agents Chemother.*, **1994**, *38*, 499–503.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 20 μL (?) 30 μg/mL pipemidic acid + 250 μL 25% sodium sulfate, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the organic layer and add it to 200 μL 1 M NaOH, shake quickly for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274 (?)

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of quantitation: 50 ng/mL

KEY WORDS
pharmacokinetics; serum

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N. Pharmacokinetics of oral fleroxacin in male and premenopausal female volunteers, *Antimicrob.Agents Chemother.*, **1996**, *40*, 789–791.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Plasma. 100 μL Plasma + 25 μL MeCN:70% perchloric acid 80:20 containing IS, mix vigorously, centrifuge. Inject a 5 or 50 μL aliquot. Dialysate. Dilute 20 μL dialysate with 980 μL 50 mM sodium dihydrogen phosphate buffer containing IS. Mix thoroughly. Inject a 5 or 10 μL aliquot.

HPLC VARIABLES
Column: Spherisorb ODS II
Mobile phase: MeCN containing 2 mM tetrabutyl ammonium hydrogen sulfate:100 mM citric acid buffer containing 5 mM ammonium perchlorate 87:13, adjusted to pH 2.2
Flow rate: 1.2
Injection volume: 5-50
Detector: F ex 290 em 460

CHROMATOGRAM
Retention time: 6.6
Internal standard: pipemidic acid (3.2)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Uehlinger,G.E.; Schaedeli,F.; Kinzig,M.; Sörgel,F.; Frey,F.J. Pharmacokinetics of fleroxacin after multiple oral dosing in patients receiving regular hemodialysis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1903–1909.

SAMPLE
Matrix: blood, intestinal efflux
Sample preparation: Intestinal efflux. Freeze intestinal efflux at -80°, lyophilize, reconstitute with 1 mL ofloxacin in MeOH:100 mM phosphoric acid 50:50, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot. Serum. Deproteinize serum with MeOH containing ofloxacin, extract with dichloromethane at pH 7.5.

HPLC VARIABLES
Column: 150 × 3.9 Novapack C18
Mobile phase: MeCN:buffer 16:84 (Buffer was 10 mM pH 3.0 potassium phosphate buffer containing 25 mM sodium heptanesulfonate (PIC B7) and 20 mM triethylamine.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 290 em 430

CHROMATOGRAM
Internal standard: ofloxacin
Limit of detection: 10 ng/mL

KEY WORDS
serum; rat

REFERENCE
Rubinstein,E.; Dautrey,S.; Farinoti,R.; St.Julien,L.; Ramon,J.; Carbon,C. Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 99–102.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma + 100 μL 1 μg/mL IS1 in 10 mM HCl + 1 mL pH 7.5 Sörensen buffer + 7 mL dichloromethane:isopropanol 70:30, extract by turning head over head at 70 rotations/min for 10 min, centrifuge at 2000 g for 10 min. Remove 5 mL of the organic

layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 500 μL mobile phase, vortex, inject an aliquot. Urine. 500 μL Urine + 100 μL 1 mg/mL IS2 in 10 mM HCl + 500 μL 1 M acetic acid + 500 μL 25 mM sodium dodecyl sulfate in water + 7 mL dichloromethane:isopropanol 70:30, extract by turning head over head at 70 rotations/min for 10 min, centrifuge at 2000 g for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 500 μL mobile phase, vortex, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK-Gel ODS 120T (Toyo Soda)
Mobile phase: MeOH:5 mM tetrabutylammonium hydrogen sulfate 21:79 (plasma) or 24.5:74.5 (urine)
Column temperature: ambient (urine), 27° (plasma)
Flow rate: 0.8
Detector: F ex 290 em 450

CHROMATOGRAM

Retention time: 7.5 (plasma), 9 (urine)
Internal standard: IS1 6-fluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (12), IS2 pipemidic acid (7)
Limit of quantitation: 10-20 ng/mL

KEY WORDS

plasma; protect from light; pharmacokinetics

REFERENCE

Dell,D.; Partos,C.; Portmann,R. The determination of a new trifluorinated quinolone, fleroxacin, its N-demethyl, and N-oxide metabolites in plasma and urine by high performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1988**, *11*, 1299–1312.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 1 mL 50 ng/mL IS1 in MeCN:water 95:5, vortex, centrifuge at 14° at 1000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 38°, reconstitute the residue in 200-400 μL mobile phase, inject a 20-30 μL aliquot onto column A and column B in series, after 1.5 min remove column A from the circuit and backflush it to waste, elute column B with mobile phase and monitor the effluent. Urine. 100 μL Urine + 100 μL 100 μg/mL IS2 in water + 5 mL mobile phase, inject a 20 μL aliquot onto column A and column B in series, after 1.5 min remove column A from the circuit and backflush it to waste, elute column B with mobile phase and monitor the effluent.

HPLC VARIABLES

Column: A 10 × 4 Nucleosil 5-C18 (replaced every 60-80 samples); B 250 × 4.6 5 μm TSK ODS-120T (Toyo Soda)
Mobile phase: MeOH:buffer 28:72, pH adjusted to 2.6 with 40% phosphoric acid (Buffer was 50 mM KH_2PO_4 containing 10 mM tetrabutylammonium hydrogen sulfate.)
Column temperature: 28
Flow rate: 1
Injection volume: 20-30
Detector: F ex 290 em 450

CHROMATOGRAM

Retention time: 7.7
Internal standard: IS1 6-fluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid (AM-735) (13.0), IS2 pipemidic acid (6.0)
Limit of detection: 1 ng/mL (plasma)
Limit of quantitation: 1000 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: ciprofloxacin, norfloxacin, pefloxacin

KEY WORDS
plasma; column-switching; protect from light; pharmacokinetics

REFERENCE
Heizmann,P.; Dell,D.; Eggers,H.; Gora,R. Determination of the new fluoroquinolone fleroxacin and its N-demethyl and N-oxide metabolites in plasma and urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *527*, 91–101.

SAMPLE
Matrix: cells
Sample preparation: Incubate cells in 2 mL 100 mM pH 3.0 glycine-HCl buffer for 2 h at room temperature, centrifuge at 5600 g for 5 min, inject an aliquot.

HPLC VARIABLES
Column: Bondapak C18
Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 25:75
Flow rate: 1.5
Detector: F ex 340 em 425

OTHER SUBSTANCES
Also analyzed: ciprofloxacin, lomefloxacin, norfloxacin, ofloxacin, temafloxacin

REFERENCE
Pascual,A.; Garcia,I.; Conejo,M.C.; Perea,E.J. Fluorometric and high-performance liquid chromatographic measurement of quinolone uptake by human neutrophils, *Eur.J.Clin.Microbiol.Infect.Dis.*, **1991**, *10*, 969–971.

SAMPLE
Matrix: milk
Sample preparation: Milk + pipemidic acid + phosphate buffer, extract with dichloromethane: isopropanol 70:30, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, add n-hexane, mix thoroughly, centrifuge, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS-120T (Toyo Soda)
Mobile phase: MeOH:5 mM tetrabutylammonium hydrogen sulfate 28:72
Flow rate: 0.8
Detector: F ex 290 em 450

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of quantitation: 100 ng/mL

KEY WORDS
protect from light; pharmacokinetics

REFERENCE
Dan,M.; Weidekamm,E.; Sagiv,R.; Portmann,R.; Zakut,H. Penetration of fleroxacin into breast milk and pharmacokinetics in lactating women, *Antimicrob.Agents Chemother.*, **1993**, *37*, 293–296.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 3 μm ODS-Hypersil
Mobile phase: MeOH:THF:670 mM pH 3.0 phosphate buffer 20:0.8:79.2 plus 2 g/L tetrabutylammonium hydrogen sulfate and 2 mL/L 85% phosphoric acid
Flow rate: 1
Injection volume: 20

Detector: UV 278

CHROMATOGRAM
Retention time: 4.94

OTHER SUBSTANCES
Simultaneous: photodegradation products, ofloxacin
Interfering: ciprofloxacin

REFERENCE
Tiefenbacher,E.M.; Haen,E.; Przybilla,B.; Kurz,H. Photodegradation of some quinolones used as antimicrobial therapeutics, *J.Pharm.Sci.*, **1994**, *83*, 463–467.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, enoxacin, norfloxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 20 μL 30 μg/mL pipemidic acid + 250 μL 500 mM pH 7.5 phosphate buffer, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the lower organic layer and add it to 200 μL 1/15 M pH 12.5 sodium phosphate, shake fast for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

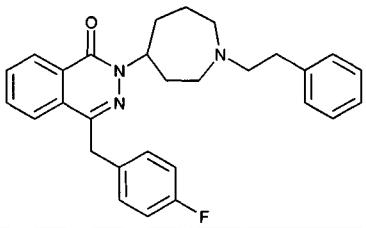
HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of detection: 50 ng/mL

KEY WORDS
pharmacokinetics

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N.; Wong,M.; Stragand,L.; Puleo,C. Effect of a fat- and calcium-rich breakfast on pharmacokinetics of fleroxacin administered in single and multiple doses, *Antimicrob.Agents Chemother.*, **1994**, *38*, 499–503.

Flezelastine



Molecular formula: $C_{29}H_{30}FH_3O$
Molecular weight: 416.58
CAS Registry No.: 135381-77-0

SAMPLE
Matrix: microsomal incubations
Sample preparation: Adjust pH of 750 μL microsomal incubation to 10 with 100 mM NaOH, add 3 mL cyclohexane:ethyl acetate 80:20, shake mechanically for 10 min, centrifuge at 2500 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with a solution of azelastine in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher Si-60
Column: 250 × 4 5 μm LiChrospher Si-60
Mobile phase: MeOH containing 0.033% perchloric acid
Flow rate: 0.5 for 17 min then 0.9
Injection volume: 20
Detector: F ex 210 em 360

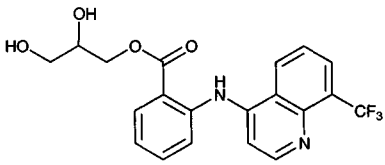
CHROMATOGRAM
Retention time: 15
Internal standard: azelastine (27)
Limit of detection: 125 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; cow; pig; liver

REFERENCE
Paris,S.; Blaschke,G.; Locher,M.; Borbe,H.O.; Engel,J. Investigation of the stereoselective in vitro metabolism of the chiral antiasthmatic/antiallergenic drug flezelastine by high-performance liquid chromatography and capillary zone electrophoresis, *J.Chromatogr.B*, **1997**, *691*, 463–471.

Floctafenine



Molecular formula: $C_{20}H_{17}F_3N_2O_4$
Molecular weight: 406.36
CAS Registry No.: 23779-99-9
Merck Index: 4140
Lednicer No.: 3 184

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 353

CHROMATOGRAM

Retention time: 10.73
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

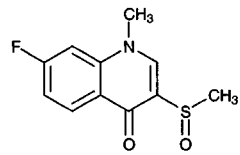
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 17.177

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Flosequinan



Molecular formula: $C_{11}H_{10}FNO_2S$
Molecular weight: 239.27
CAS Registry No.: 76568-02-0
Merck Index: 4146
Lednicer No.: 5 127

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 µL 100 µg/mL IS in MeCN:MeOH 50:50 + 5 mL chloroform, shake for 10 min, centrifuge at 1800 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Chiralcel OD
Mobile phase: MeOH:EtOH 78:22
Column temperature: 30
Flow rate: 0.7
Detector: UV 320

CHROMATOGRAM
Retention time: 12.1 (R(+)), 7.9 (S(-))
Internal standard: (±)-7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (8.9, 14.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Kashiyama,E.; Odomi,M.; Shimizu,T. Stereospecific and simultaneous high-performance liquid chromatographic assay of flosequinan and its metabolites in human plasma, *J.Chromatogr.B*, **1994**, *652*, 179–185.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 1 mL Baker-10 octadecyl C18 SPE cartridge (J.T. Baker) with MeOH then water. 100 μL Plasma or urine + 25 μL 2 (plasma) or 10 (urine) μg/mL IS in water, add to the SPE cartridge, wash with 2 column volumes of water, elute with 200 μL MeOH, inject a 2 (urine) or 5 (plasma) aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:water 7:20:73
Flow rate: 1.2
Injection volume: 2-5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4
Internal standard: 7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (7.8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, amiodarone, diazepam, digoxin, furosemide, lidocaine, nitroglycerin, procainamide, propranolol, quinidine

KEY WORDS
plasma; SPE

REFERENCE
Slegowski,M.B.; Miller,C.; Porter,R.S. Simplified high-performance liquid chromatographic determination of flosequinan and its metabolite in plasma, serum and urine, *J.Chromatogr.*, **1988**, *425*, 227–232.

SAMPLE
Matrix: incubations
Sample preparation: 2 mL Incubation + 10 μg IS + 4 mL chlorobutane:1,2-dichloroethane 80:20, shake for 15 min, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 800 μL mobile phase, inject a 100 μL aliquot.

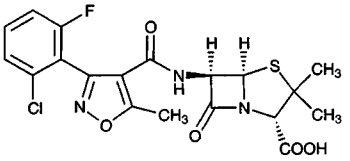
HPLC VARIABLES
Column: 150 × 4.6 Hypersil 3ODS
Mobile phase: MeCN:MeOH:water 7:20:73
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: 7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (BTS 49037)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat intestinal flora and by *Escherichia coli in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1567–1576.

Floxacillin



Molecular formula: $C_{19}H_{17}ClFN_3O_5S$
Molecular weight: 453.88
CAS Registry No.: 5250-39-5, 1847-24-1 (sodium salt), 34214-51-2 (sodium monohydrate)
Merck Index: 4147

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 μL Plasma + 100 μL 20 μg/mL dicloxacillin sodium in water, add 400 μL MeCN at -15° while vortexing, add 700 μL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 μL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 2 5 μm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with ortho-phosphoric acid
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: dicloxacillin (3.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: cloxacillin

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL dicloxacillin in water + 25 μL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220